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Resistin hormone level among Type 2 Diabetic Patients in Gaza Governorate

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgement has been made in the text.

Signature **Date**

Monis M. E Hendy *August 2013*

Dedication

To my Parents, **Dr. Marwan & Mrs. May** the source of guidance and inspiration, who have been supporting me on the way of success.

To my wife **Mrs. Shoroq**, the light of my life, my colleagues in this master program who has encouraged me strongly to take the first step towards post graduation.

To my father & mother in law **Mr. Rafat & Mrs. Wafaa** who have been paying a lot of efforts to let me success in this work.

To my second mother, **Aunt Mona, my** beloved brother **Eng. Mohamed** & my beloved sister **Maysaa**.

To the hope of my life my son **Baraa** who is giving me the power to keep on & to continue.

Dedication is almost expressed to the Palestinian people who are suffering and struggling with the persistence to have a free Palestine.

Monis Ell Hendy

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Resistin hormone level among type 2 Diabetic patients in Gaza Governorate

Abstract

Objective: To assess resistin hormone among type 2 males diabetic patients (T2DP) in Gaza Governorate.

Subjects and methods: It is a case control study included 90 males subjects, 45 of them were T2DP and the rest of them were 45 healthy non diabetic controls that matched each other in ages, sex and residence. A questionnaire interview was used as a tool of study. Serum resistin, glucose, insulin, cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and homeostasis model assessment of insulin resistance (HOMA –IR) were determined. Data were analyzed using SPSS version 18.0.

Results: The mean ages of controls and cases were 50.93±5.74 and 51.64±4.76 years, respectively. Diabetes mellitus was more prevalent among individuals with family history of diabetes. Most of studied patients had had diabetes since less than 5 years.

The self-reported complications among diabetic patients were hypertension, numbness in toes, heart diseases and retinopathy. Smoking or Physical activity had no association with serum resistin level (P>0.05). The BMI hadn't also any possible correlation with serum resistin level. The mean level of serum resistin had not any significant difference between diabetic patients and controls (P>0.05). Moreover the mean serum glucose levels in cases were significantly higher than that in controls (180.78±76.3 mg/dl Vs 90.89±12.88 mg/dl) (P=0.000) with no any significant correlation between resistin with glucose. The mean serum insulin an insulin resistant levels in cases were significantly

higher than that in controls (P>0.05). The average levels of cholesterol, triglycerides, HDL-C and LDL-C had not significantly difference in cases compared to controls. The Pearson correlation test revealed negative significant correlation between resistin and triglyceride or cholesterol (P<0.05).

Conclusion: The preliminary study revealed that Serum resistin hasn't any significant relation with type 2 diabetes mellitus or insulin resistant.

Keywords: Resistin hormon, Insulin hormone, Insulin resistant, Diabetes mellitus type 2, Gaza, IUG.

تقييم مستوى هرمون الريسيستين لدى مرضى السكري النوع الثاني في محافظة غزة

مستخلص الدراسة

الاهداف : تقييم مستوى هرمون الريسيستين لدى مرضى السكري النوع الثاني من الذكور في محافظة غزة.

الطرق والادوات : قامت هذه الدراسة على منهجية بحث (مجموعة مرضية-مجموعة ضابطة). المجموعة المرضية تتكون من 45 مريضا من مرضى السكري النوع الثاني من الذكور والمجموعة الضابطة تتكون من 45 متطوع من الاصحاء غير المصابيين بمرض السكري. تم التطابق بين المجموعتين في الجنس و العمر والاقامة في غزة. وقد تم الحصول على نتائج من خلال المقابلة الشخصية واجراء الفحوصات البيوكيميائية لكل من الريسيستين و الجلوكوز والانسولين و الكوليسترول و الدهون الثلاثية و الدهون البروتينية ذات الكثافة المرتفعة و الدهون البروتينية ذات الكثافة المنخفضة و تقييم نموذج التوازن لمقاومة الانسولين.

النتائج : متوسط العمر للمجموعة المرضية 50.93±5.74 سنة ومتوسط العمر للمجموعة الضابطة 51.64±4.76 سنة. مرض السكري اكثر انتشارا بين الاشخاص الذين لهم تاريخ عائلي للمرض، و معظم مرضى السكر كانوا مصابين بالمرض منذ اقل من 5 سنوات. تراوحت نسبة المضاعفات الذاتية لمرض السكري بين ارتفاع ضغط الدم و تنميل الاصابع و امراض القلب و اعتلال شبكية العين. أشارت النتائج الى أنه لا يوجد علاقة بين التدخين أو النشاط البدني ومستوى هرمون الريسيستين. ولم تثبت أيضا علاقة بين مؤشر كتلة الجسم و مستوى هرمون الريسيستين، و لا يوجد اختلاف في متوسط مستوى هرمون الريسيستين بين المجموعة المرضية والمجموعة الضابطة، واما مستوى الجلوكوز وهرمون الريسيستين ومقاومة الانسولين فقد كان مرتفعا في مرضى السكري عنه في المجموعة الضابطة، ولم تثبت الدراسة علاقة بين الجلوكوز أو

الانسولين او مقاومة الانسولين مع مستوى هرمون الريسيستين، و لم يختلف مستوى الكوليسترول والدهون الثلاثية والدهون البروتينية ذات الكثافة المرتفعة والدهون البروتينية ذات الكثافة المنخفضة في مرضى السكري عنه في المجموعة الضابطة، بينما كان هناك علاقة بين الكوليسترول والدهون الثلاثية مع مستوى هرمون الريسيستين.

الاستنتاج : اظهرت هذه الدراسة الأولية انه لا علاقة بين هرمون الريسيستين ومرض السكري من النوع الثاني او مقاومة الانسولين .

الكلمات المفتاحية : هرمون الريسيستين، هرمون الانسولين، مقاومة الانسولين، مرضى السكري النوع الثاني ، قطاع غزة، الجامعة الاسلامية.

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AGE	Advanced glycated end product
BM I	Body mass index
CAD	Coronary artery disease
DHEA	Dehydroepialdrosterone
DM	Diabetes mellitus
ELISA	Enzyme-linked Immunosorbent Assay
FFA	Free fatty acids
GLUT4	Glucose transporter 4
HDL	Height density lipoprotein
HOMA-IR	Homeostasis model assessment for insulin resistance
IGF-1	Insulin growth factor-1
IL	Interleukin-6
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1
LDL	Low density lipoprotein
MRS	Magnetic resonance spectroscopy
PA	Physical activity
PPAR-γ	peroxisome proliferator-activated receptors gamma

PPREs	peroxisome proliferator response elements
RSG	Rosiglitazone
T2DM	Type 2 Diabetes mellitus
TG	Triglyceride
TNFa	Tumor necrosis factor alpha
TZDs	Thiazolidinediones
VLDL	Very low density lipoprotein
WAT	White adipose tissue
WHO	The world health organization

Chapter 1

Introduction

1.1 Overview

Diabetes mellitus is the most common endocrine disorder in human, currently affecting over 170 million people world-wide and, potentially, over 365 million in the year 2030 **(Saini, 2010).** Diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both **(WHO, 1999).** There are several distinct types of diabetes exist, but two most common types are type I diabetes and type 2 diabetes **(Kota et al., 2012**).

Type 2 diabetes accounts for 75% of all diabetes cases **(Ehtesham, 2001)**. It is one of the most prevalent chronic diseases in the world, which is characterized by insulin resistance coupled with a failure of pancreatic cells to compensate by adequate insulin secretion **(Zhanga et al., 2010).**

The risk factors for T2DM include environmental influences such as obesity (especially abdominal obesity), aging, ethnicity, family history of diabetes, history of gestational diabetes, sedentary lifestyle, low birth weight, and polycystic ovary syndrome **(Moneva & Dagogo-Jack., 2002).** Eighty percent or more of the people with T2DM are obese with the remaining twenty percent considered above ideal weight indicating obesity as a predominant link to the development of T2DM **(Kahn & Flier., 2000).**

Insulin is a small globular protein containing two chains, A (21 residues) and B (30 residues) **(Weiss et al., 2009).** It is synthesized exclusively in pancreatic islet B-cells by various stimuli, and gene mapping studies assigned the human insulin gene to chromosome 11 **(Watanabe, 2011).** Insulin is a pleiotropic hormone that exerts a multitude of effects on metabolism and various cellular processes in different tissues and organs of the body. The main metabolic actions of insulin are to stimulate glucose uptake in skeletal muscle and the heart and to suppress the production of glucose and very-low density lipoprotein (VLDL) in the liver **(Jellinger et al., 2007).** Other metabolic effects include inhibition of glucose release from the liver, inhibition of the

release of free fatty acids (FFAs) from adipose tissue, and stimulation of the process by which amino acids are incorporated into protein **(Eckel et al., 2005).**

The first clinical evidence relating IR hyperandrogenism dates back to 1921 when Achard and Thiers described the existence of women with glucose intolerance and hirsutism ("the bearded diabetic woman**"**) **(Espinós & Alsina., 2005)**. Insulin resistance is a characteristic of human obesity, and a primary risk factor for the (T2DM). However, despite tremendous effort and great progress in a number of areas of research, the biochemical mechanisms underlying the pathogenesis of insulin resistance in obesity remain poorly understood **(Bhatt et al., 2006)**.

Obesity is a worldwide health problem directly linked to several disease processes such as hypertension and type 2 diabetes mellitus **(Liu et al., 2008**). Obesity is generally defined as excessive body fat that results in increased risk for morbidity and mortality **(Seo et al., 2012).** Obesity occurs when energy intake exceeds energy expenditure. Our evolutionary history led to selection for consumption of high fat, protein rich and calorie dense food in times of plenty to protect against famine. The relatively recent availability of highly palatable, and energy dense food, combined with more sedentary lifestyles, have contributed to the global obesity epidemic **(Chen & Morris., 2007).** However, obesity is considered the most important risk factor for type 2 diabetes, as obese individuals are seven times more likely to develop than are normal-weight individuals. In addition, central obesity is strongly correlated with insulin resistance in type 2 diabetic patients **(Gharibeh et al., 2010).**

Adipose tissue is an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, inflammation and immunity. Adipocytokines are proteins produced mainly by adipose tissue. These molecules have been shown to be involved in the pathogenesis of the metabolic syndrome and cardiovascular disease, For example, adiponectin, leptin, TNF alpha, interleukin and resistin **(Luis et al., 2009).**

Resistin is a novel 12.5 kDa cysteine-rich polypeptide secreted from adipocytes in rodents and humans. Resistin antagonizes insulin-stimulated glucose metabolism in muscle myocytes, hepatocytes, and adipocytes themselves **(Zhong et al., 2002).** However, the role of resistin in response to obesity and insulin resistance in type 2 diabetic patients is still obscure. Several studies have reported increased resistin levels in association with obesity and insulin resistance in T2DM, whereas other studies have failed to detect any change in resistin levels under such conditions **(Gharibeh et al., 2010).** Yet other studies found that circulating resistin levels are involved in promoting adiposity, but had no effect on the degree of insulin resistance **(Laudes et al., 2010).**

1.2 General objective

- To assess resistin hormone among type 2 male diabetic patients in Gaza strip.

1.3 Specific objectives

- To study resistin hormone among study population.
- To measure insuline hormone, blood glucose and lipid profile among study population.
- To verify possible relations between resistin hormone and anthropometric measures.

1.4 Significance

The prevalence rate of DM in Palestine is about 9% in 2000 **(MOH, 2004).** A new hormone which has not been studied before in Gaza strip is resistin. However, a lot of studies were carried out about resistin relation with obesity and insulin resistance in type 2 diabetes mellitus in many countries **(Steppan et al., 2001 & Kusminski et al., 2005)**, their results were controversial and most of them recommended to make further studies about resistin among different races and different gene population.

Chapter 2

Literature review

2.1 Diabetes mellitus

Diabetes is a lack of insulin or presence of factors opposing the action of insulin, resulting in an increase (hyperglycemia) in blood glucose levels **(Ehtesham, 2001).** Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, Blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketone hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made **(WHO, 1999).** T2DM is a multistage process that begins as insulin resistance, characterized by inability of the body to use its own insulin properly, and ends with exhaustion of the insulin producing pancreatic cells, thereby leading to hyperglycemia **(Gharibeh et al., 2010).**

2.2 Pathophysiology of type 2 diabetes Mellitus

2.2.1 Insulin hormone

Insulin is a hormone required to facilitate transport of blood glucose across the largely impermeable cell membrane. It is released in response to elevations in blood glucose. The insulin binds to a receptor on the cell membrane, allowing the entry of glucose into muscle and fat cells to form glycogen, fatty acids to generate triglycerides, and amino acids for protein synthesis. It is thus an anabolic hormone. It is a potent stimulator of growth factors, including insulin growth factor 1 (IGF-1). It also inhibits catabolic processes such as the breakdown of glycogen and fat, and decreases gluconeogenesis **(Cosford, 1999).**

2.2.2 Insulin sensitivity

Insulin sensitivity refers to the ability of insulin to support glucose homeostasis by signaling insulin-sensitive tissues or organs to absorb glucose. These signals include stimulating glucose utilization in both muscle and adipose tissue and suppressing the

production of glucose by the liver. Both responses act to decrease plasma glucose concentration. The degree of impairment of glucose metabolism is influenced both by the insulin sensitivity of cells within the body and by pancreatic cell reserve capacity **(Jellinger et al., 2007).**

2.2.3 Insulin resistance (IR)

IR is a critical feature of T2DM that may be detected 10–20 years before the clinical onset of hyperglycemia **(Zhao & Townsend., 2009).** IR is present in the non-diabetic offspring of patients with type 2 diabetes; prospective studies demonstrating the usefulness of IR as a predictive marker of the future development of T2DM and prevention of diabetes by insulin sensitizing agents **(Savage et al., 2005).**

Insulin resistance is defined as a failure of target tissues (adipose, liver, skeletal and cardiac muscle) to respond normally to insulin. At the molecular level, this resistance can occur anywhere in the insulin signaling pathway, from receptor binding to downstream signaling events **(Steppan & Lazar., 2002)**. Under this condition, the islet β-cells in the pancreas secrete higher levels of insulin to compensate for the declined receptor function making hyperinsulinaemia a common characteristic in T2DM patients **(Zhao & Townsend,. 2009).** Although standard definitions of IR still define it in terms of the effects of insulin on glucose metabolism, the last decade has seen a shift from the traditional "glucocentric" view of diabetes to an increasingly acknowledged "lipocentric" viewpoint. This hypothesis holds that abnormalities in fatty acid metabolism may result in inappropriate accumulation of lipids in muscle, liver, and β-cells **(savage et al., 2005).**

2.2.4 Insulin resistance mechanism

Under hyperglycemic and hyperinsulinemic conditions, muscle glycogen synthesis is the major pathway for glucose metabolism in both normal and diabetic individuals, and that defective muscle glycogen synthesis plays a major role in causing insulin resistance in patients with T2DM **(Shulman, 2000).** Using multinuclear MRS approach, it was able to determine that glucose transport was the rate controlling step for insulin-stimulated muscle glycogen synthesis in T2DM, rather than hexokinase. Therefore, glucose

transport represents the best target to correct insulin resistance in skeletal muscle in patients of T2DM **(Morino et al., 2006).**

Glucose-6-phosphate is an intermediate between glucose transport into the cell and its subsequent glycogen synthesis. The increment in glucose-6-phosphate concentration was significantly reduced in T2DM, suggesting that glucose transport or phosphorylation must be the rate-controlling step in insulin-stimulated glucose disposal in skeletal muscle rather than glycogen syntheses as seen in (figure2.1) **(Savage et al., 2005).**

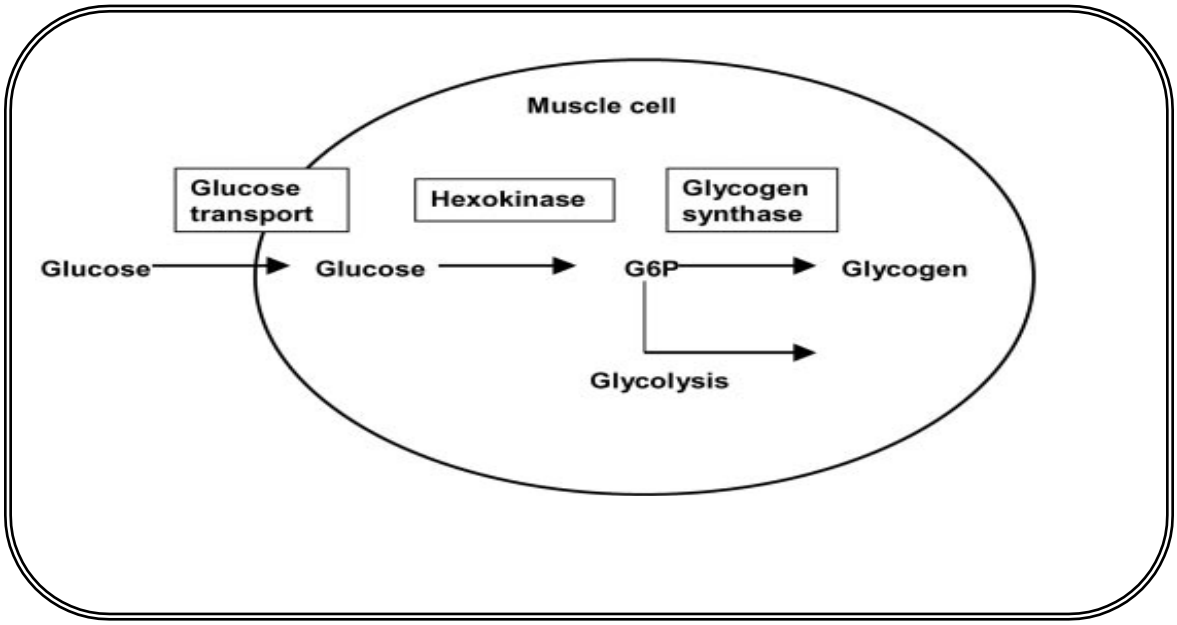

Figure 2.1 Potential rate-controlling steps in insulin-stimulated glycogen synthesis in a myocyte. (Savage et al., 2005).

2.2.5 Fatty acid–induced insulin resistance

Increased plasma free fatty acid concentrations are typically associated with many insulin-resistant states, including obesity and T2DM **(Shulman et al., 2000).** Free fatty acids (FFAs) released from adipose tissue increase production of glucose and triglycerides (TGs) and secretion of very-low-density lipoprotein (VLDL) in the liver. Associated lipid/lipoprotein abnormalities include decreased levels of high-density lipoprotein cholesterol (HDL-C) and increased levels of small dense low-density lipoprotein (LDL) particles. FFAs also reduce insulin sensitivity in muscle by inhibiting

insulin mediated glucose uptake. Increases in circulating glucose increase pancreatic insulin secretion, resulting in hyperinsulinemia. Hyperinsulinemia may result in enhanced sodium reabsorption from kidney and increased sympathetic nervous system activity. It also may contribute to the development of hypertension see figure (2.2) **(Jellinger et al., 2007).**

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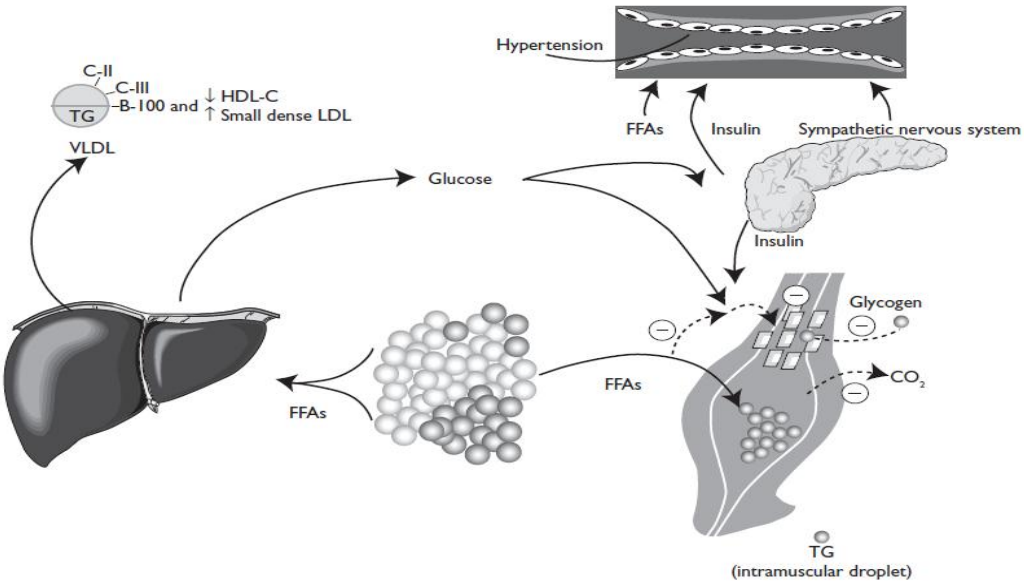


Figure 2.2 Pathophysiology of insulin resistance (Jellinger et al., 2007).

Savage et al., (2005) originally showed that fatty acids compete with glucose for substrate oxidation in isolated rat heart muscle and rat diaphragm muscle. The mechanism they proposed to explain the insulin resistance was that an increase in fatty acids caused an increase in the intra-mitochondrial acetyl CoA/CoA and NADH/NAD+ ratios, with sub- sequent inactivation of pyruvate dehydrogenase (Figure 2.3). This in turn would cause intracellular citrate concentrations to increase, leading to inhibition of phosphofructokinase, a key rate-controlling enzyme in glycolysis. Subsequent accumulation of glucose-6- phosphate would inhibit hexokinase II activity, resulting in an increase in intracellular glucose concentrations and decreased glucose uptake. **(Shulman., et al 2000).**

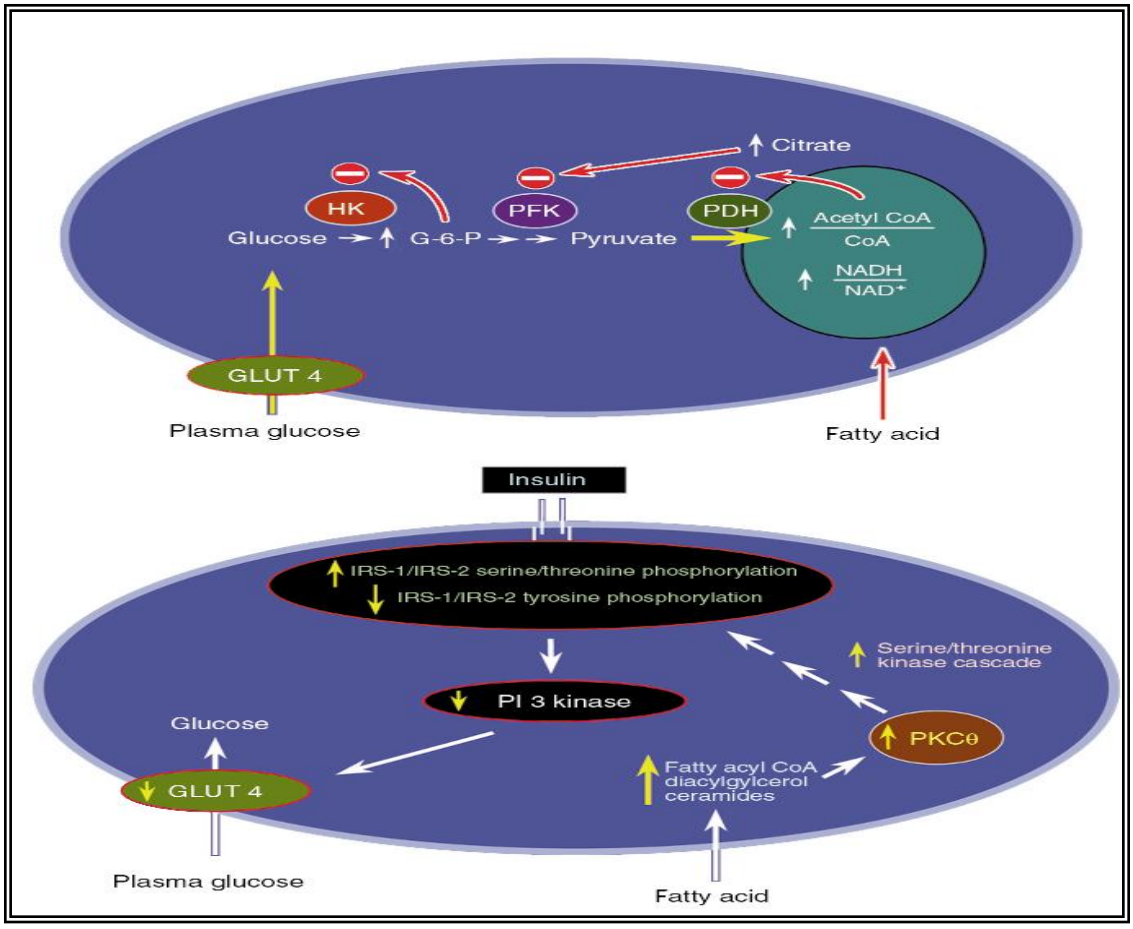


Figure 2.3 Mechanism of fatty acid–induced insulin resistance in skeletal muscles (Shulman., et al 2000).

Lipid accumulation within skeletal muscle and liver inhibits tyrosine phosphorylation of IRS-1. In turn, this follows serine phosphorylation of critical sites on IRS-1 and inhibits binding and activation of PI 3-kinase. A number of different serine kinases could be responsible for serine phosphorylation of IRS-1. Candidates include members of the novel protein kinase C family, which may be activated by accumulation of lipid intermediates, as well as inflammatory intermediates. The latter may be activated within adipose tissue in obese states. Lipid accumulation in skeletal muscle and liver may be a result of increased delivery/synthesis of fatty acids to/in these tissues in states in which energy intake exceeds adipose tissue storage capacity (as seen in obesity and lipodystrophy) or a consequence of either acquired or inherited mitochondrial dysfunction. **(savage et al., 2005).**

2.3 Obesity:

Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measured of obesity is the body mass index (BMI), with a BMI of 30 or more considered obese and BMI equal to or more than 25 considered overweight. Obesity has become a global epidemic, with more than 1 billion overweight adults and at least 300 million obese patient's worldwide **(Chiang et al., 2011)**.

BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults but it is merely a rough guide because it may not correspond to the same degree of fatness in different individuals **(Gupta et al., 2010).**

Adipose tissue plays a crucial role in the regulation of whole-body fatty acid homeostasis. In periods of calorie abundance it stores free fatty acids (FFAs) in the form of triglycerides through their esterification to glycerol and releases them back into the circulation in times of energy shortage **(Galica., et al 2010).** Obesity-induced insulin resistance is affected both by the total amount of adipose tissue and its distribution. Both visceral and deep subcutaneous adipose tissues are associated with insulin resistance. Excessive free fatty acids (FFAs) released by lipolysis from adipose tissue have been implicated in non-insulin dependent diabetes mellitus. FFAs compete with glucose for oxygen and inhibit whole body glucose disposal via the 'Randle cycle'. FFAs have a deleterious effect on insulin uptake by the liver and contribute to the increased hepatic glucose release **(Steppan & Lazar., 2002).** Adipose tissue is not only an organ for passive energy reserve, but also an active endocrine organ secreting a wide range of hormones and other protein factors called adipokines **(Liu et al., 2008).**

2.4 Resistin hormone

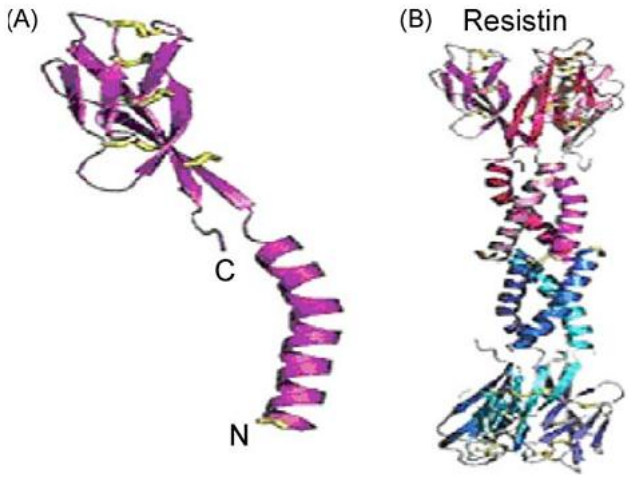
Resistin, an adipocyte-derived hormone, which is associated with insulin resistance in vivo and in vitro, has been considered to link with T2DM. It serves as a signaling molecule between the energy storage organ, adipose tissue, and the principal insulin-responsive organs: liver, muscle and fat and is considered to be a major contributor to insulin resistance in obesity **(Steppan et al., 2001).**

2.4.1 Discovery of resistin

A new gene family named resistin-like molecules was discovered at almost the same time by different research groups. Initial studies showed that resistin was up-regulated in rodent models of obesity and insulin resistance and down-regulated by an insulin sensitizer RSG (rosiglitazone); however, Immunoneutralization of resistin reduced hyperglycemia and improved insulin sensitivity. **(Kusminiski et al., 2005).**

2.4.2 The structure of resistin

The human peptide consists of 114 amino acids including a 17 amino acids 61 signal peptides, a variable region of 37 amino acids, and a conserved C-terminus **(Korner et al ., 2005).** The cysteine-rich peptide has molecular weight of 12.5 Kda and circulates in human plasma as a dimeric protein at levels ranging between 2.5 and 21.5 ng/ml **(Silha et al., 2003).** Variations in plasma resistin levels have been studied and its level appears to be fairly stable throughout the day. This is partly because of the fact that resistin does not seem to be regulated by eating and it has been seen that 48-h fasting did not affect plasma resistin levels. In addition, the level of resistin did not correlate with total energy see figure (2.4) **(Gupta et al., 2010).**



Figur 2.4 Ribbon diagram representations of resistin.

(A) Monomeric structure of resistin, whereas (B) shows its hexameric form comprising of two disulphide-linked trimers. (C) Highly exposed interchain disulphide present in the hexameric form of resistin **(Kusminisky et al., 2005)**

2.4.3 Tissue distribution of resistin and other resistin-like molecule family members

Resistin expression was first described in adipose tissue, with circulating levels detected in rodents and humans. Although the expression of resistin in mice was originally restricted to adipocytes, the principle origin of human resistin has remained somewhat contentious. Unlike the mouse gene, the human homologue of resistin was sparsely detectable in human adipocytes, this was confounded further by confusion over the proposed sites of resistin production. These studies led to many of the current perceptions that resistin was an inconsequential factor in the progression of obesity related T2DM, thereby contrasting rodent data (**Kusminski et al., 2005)**. Conversely, McTernan et al, detected resistin in adipose tissue, thus describing resistin as a potential pathogenic factor increased in central adiposity **(Mcternan et al., 2003).** The discrepancy between studies may have partially related to methodology of detection or quantification of resistin. However, to date, although a difference in resistin mRNA levels between adipocytes and macrophages is apparent, studies have not yet identified whether this difference is observed at the protein level. Determining the relative contribution of the adipocyte in obesity with regards to circulating resistin levels would also prove beneficial **(Kusminski et al., 2005)**.

2.4.4 Regulation of resistin

Resistin is tightly controlled by nutritional and hormonal conditions. Resistin is low in fasting conditions in parallel with glucose and insulin, and is restored upon re-feeding. Insulin appears to suppress resistin gene expression in 3T3-L1 adipocytes, 83 and hyperglycemia promotes resistin expression. Insulin-like growth factors, that are 84 known to stimulate adipogenesis, down regulate resistin gene expression. The tissue level of resistin is decreased by insulin and cytokines such as TNFa, endothelin-1 and dexamethasone, and increased by growth and gonadal hormones and DHEA, hyperglycemia, male gender, and some pro inflammatory cytokines, such as IL-6 and lipopolysaccharide **(Korner et al., 2005)**.

2.4.5 Regulation of resistin in models of insulin resistance and glucose intolerance

In evaluating resistin and its association with insulin sensitivity in humans, several studies have identified positive correlations between resistin levels and insulin resistance *in vivo* and *in vitro*. Additionally, serum resistin levels were increased by approx. 20% in T2DM subjects, such findings have been re-affirmed by **(Fujinami et al., 2004)**. In contrast, other studies have reported no associations between serum resistin levels and markers of insulin resistance in T2DM patients or insulin-resistant patients. Moreover, serum and plasma resistin levels were either reduced or increased in T2DM patients with no significant correlation with HOMA-IR (homoeostasis model assessment for insulin resistance), waist circumference, BMI or total cholesterol **(youn et al., 2004)** and **(yang et al., 2003)** Consequently, these studies suggest resistin is unlikely to play a critical endocrine role in insulin resistance or energy homoeostasis in humans. Nevertheless, a paracrine or autocrine manner of resistin to moderately affect metabolism cannot be ruled out **(Kusminski et al., 2005)**.

In the human context, early studies showed contradictory findings for correlations between resistin and glucose disposal, with recent studies still providing inconsistent results. Studies in Pima Indians have reported serum resistin effect of resistin on glucose homoeostasis levels were not associated with fasting glucose and insulin levels, although they were proportional to the degree of adiposity **(Vozarova et al., 2004).** Additionally, one study indicated serum resistin levels were inversely correlated with glucose disposal rates, whereas others indicate a modest effect of resistin on glucose uptake *in vitro*. Collectively, resistin transgenic and gene-deletion studies in rodents have provided evidence that resistin may have a predominant physiological role in the liver by contributing to the regulation of fasting blood glucose levels. Consequently this may have important implications in humans, but further studies regarding this are clearly required **(Kusminski et al., 2005)**.

2.4.6 Regulation of resistin by insulin sensitizers

recent human studies have shed favorable light on the modulation of resistin by TZDs. Pioglitazone treatment suppressed plasma resistin concentrations in T2DM patients, which positively correlated with decreased hepatic fat content and improved insulin sensitivity. Furthermore, RSG reduced resistin secretion from human adipocytes and resistin expression in human macrophages. To understand whether the down regulation of resistin expression by RSG occurred through a direct PPAR-γ mediated transcriptional mechanism, identification of five putative PPREs (PPAR-γ -response elements) in the resistin gene. One such response element, PPRE2, was shown to bind PPAR-γ. However, how PPAR-γ exerts its suppressive actions on resistin expression remains to be elucidated, although it has been suggested that recruitment of co-repressors of transcription may play a role. RSG has also been shown to have anti-inflammatory effects in human macrophages by reducing inflammatory cytokine production, which may consequently affect resistin production. Collectively, these human studies indicate that suppression of resistin expression may contribute to the insulin-sensitizing and glucose-lowering actions of the TZDs. Furthermore, the potential anti-inflammatory effects of TZDs on adipocytokine mediation may be of equal importance in the prevention of T2DM **(Kusminski et al., 2005)**.

2.4.7 Hormone and cytokine modulators of resistin expression

Studies investigating metabolic hormones and cytokines that are associated with insulin resistance have looked for their relationship with resistin. Such studies have produced data suggesting an interplay between hormones, cytokines and resistin, as shown in Table (2.1). Although the reported regulation of resistin by these factors appears intriguing, no underlying mechanistic principles are currently apparent. Therefore the physiological relevance of most of these factors with respect to resistin remains to be determined **(Kusminski et al., 2005)**.

Table 2.1 Effects of hormones and cytokines on the level of resistin expression and secretion

Hormone/cytokine	Effect on resistin
Pituitary hormones	Growth hormone (somatotrophin; 1 mg · kg−1 · day−1) ↑ resistin gene expression (720–950 %) in WAT of spontaneous dwarf rats and has moderate inhibitory effects on resistin transcript and protein (30–50 %) levels in 3T3-L1 adipocytes; ↑ gene expression levels in response to hyperprolactinaemia in mice;↓ mRNA and protein expression (30–50 %) in 3T3-L1 adipocytes.
Steroid hormones	Dexamethasone ↑ mRNA and protein levels (2.5- to 3.5–fold) in 3T3-L1 adipocytes and approx. 70 % in mouse WAT.
Sex hormones	↑ In mice with elevated androgen levels; ↑ by hyperprolactinaemia and testosterone; administration of dehydroepiandrosterone ↑ gene expression in WAT of male Wistar rats; oestrogen ↓ adipose gene expression in ovariectomized rats and in isolated rat adipocytes in vitro; testosterone ↑ adipose tissue mRNA levels in male rats.
Thyroid hormone	Severely ↓ expression in hyperthyroid rats; ↓ serum levels in subjects with hyperthyroidism.
Adrenaline	↓ Transcript and protein levels (30–50 %) in 3T3-L1 adipocytes.
Neuropeptide Y	Intracerebroventricular administration of neuropeptide Y ↑ gene expression in mice WAT.
***β*3-Adrenoreceptors**	The *β*3-agonist isoproterenol ↓ gene expression levels in vitro by 20 % in 3T3-L1 adipocytes, which is reversible with the *β*3-antagonist propranolol.
ET-1	ET-1 (100 nM) ↓ basal secretion by 59 % in 3T3-L1 adipocytes.
Insulin	↓ Gene expression (approx. 50 %) in 3T3-L1 adipocytes; ↑ secretion from 3T3-L1 adipocytes; ↑ mRNA synthesis (23-fold) in streptozotocin-diabetic mice or Zucker diabetic fatty rats; gene expression and protein concentration ↑ in fasted mice; ↑resistin protein secretion in a concentration-dependent manner in human subcutaneous adipocytes.

(Kusminski et al., 2005).

2.4.8 Resistin and obesity

Recent investigations of human resistin in relation to obesity have shown higher serum resistin levels in obese subjects compared with lean subjects, which positively correlated with the changes in BMI and visceral fat area. The implication that resistin is important in human adipose tissue has been corroborated by studies showing increased protein expression with obesity, as well as protein secretion from isolated adipocytes. These recent observations are concomitant with initial studies that showed increased serum resistin levels and gene expression levels in abdominal depots in states of increased adiposity. A further study has shown a significant reduction in circulating resistin levels following moderate weight loss and post-gastric bypass. Collectively, these observations suggest resistin could indirectly be subjected to nutritional regulation in humans **(Kusminski et al., 2005).**

Population based studies have not clarified the role of resistin in obesity. No correlation with BMI has been reported for example in a general population of 1090 subjects without medication recruited for a study of intima-media thickness (IMT) progression **(Norata et al., 2007)** or in subjects with type 2 diabetes (n = 199) **(Youn et al. 2004).** In addition, in two large studies of 1922 and 1162 patients with or without CAD, resistin was not correlated with BMI **(Lubos et al., 2007, Pilz et al., 2007).** However, an association between resistin and obesity has been reported in almost as many papers as in those where no association was seen. The plasma resistin concentration has been associated with higher BMI in subjects with type 2 diabetes (n = 113) **(Tokuyama et al., 2007)**, non-diabetic subjects (n = 123) **(Aquilante et al., 2008),** and asymptomatic subjects with family history of premature CAD (n = 879) **(Reilly et al., 2005).**

A correlation between plasma resistin level and body fat mass or percent body fat has been reported in young 45 healthy subjects **(Yannakoulia et al., 2003),** patients positive for human immunodeficiency virus (HIV) **(Barb et al., 2005),** as well as in overweight Pima Indians, where resistin was also associated with the increase in percent body fat during an average of 4.5 year follow-up **(Vozarova de Conneely et al, . 2004).**

2.4.9 Resistin, insulin, insulin resistance and T2DM

It is currently established that central obesity is a contributing factor to the pathogenesis of insulin resistance and consequently to T2DM. Although it is apparent that inconsistencies remain in the data for a role of resistin in obesity, there is a growing body of evidence suggesting a role for resistin in the etiology of insulin resistance and T2DM **(Kusminski et al., 2005)**. Studies on the plasma resistin level in type 2 diabetes have described similarly conflicting results as the studies on obesity. There are studies that have reported higher plasma resistin concentrations in subjects with type 2 diabetes **(Al-Daghri et al., 2005, Al-Harithy & Al-Ghamdi 2005),** whereas there is an almost equal number of studies in which no such difference has been observed **(Fehmann & Heyn., 2002, Chen et al., 2006).** The association of resistin to diabetes is obscure since even those studies observing a difference in plasma resistin level between subjects with type 2 diabetes and controls have not always detected significant association between resistin and insulin resistance **(Youn et al., 2004, Hasegawa et al., 2005, Koçak et al., 2007)**. Furthermore, the largest studies (n > 500) on plasma resistin concentration with the exception of the study of Pilz and co-workers **(Pilz et al., 2007),** have not detected any independent association with insulin resistance in different study populations **(Reilly et al., 2005 & Norata et al., 2007)**.

2.4.10 Functional roles of resistin and involvement in the disease

Steppan et al. (2001) made the original suggestion that resistin represented a hormone that links obesity to diabetes. The term resistin is derived from "resistance to insulin" and the peptide was, indeed, demonstrated to counteract the beneficial effects of insulin by suppressing insulin signaling **(Kusminski et al., 2005)**. Insulin resistance and dyslipidemia were found to be associated with resistin over expression and resistin was shown to inhibit cellular glucose uptake, including by cardiomyocytes. **Silha et al. (2003)** demonstrated that plasma resistin correlated with insulin resistance in lean and obese human subjects. Other workers, however, reported that serum resistin was not a reliable predictor of insulin resistance when adjusted for BMI. Thiazoladinediones, used to treat insulin resistance in type 2 diabetes, and which are cardio protective drugs, have been shown to inhibit resistin synthesis by adipocytes and lower serum resistin **(Steppan et al., 2001)**

Chapter 3

Subjects and Methodology

3.1 Study design

It is a Case control study.

3.2 Study population

The target population was type 2 diabetic male aged 40-60 years from different diabetic clinic centers in Gaza Governorate.

3.3 Sample size

The sample size was 45 type 2 diabetic males and 45 healthy males served as controls. Patients and controls were also a matched each other in ages and residence.

3.4 Sampling

A total of 45 blood samples were collected from T2DP, which were previously diagnosed according to the current WHO diagnostic criteria for diabetes (WHO, 2006), from the diabetic clinic centers in Gaza Governorate. Blood samples were also collected from healthy persons who visited the clinic center with their relatives and were served as controls.

3.5 Exclusion criteria

1) Patients aged <40 and >60 years.
2) Females.
3) Blood diseased patients.
4) Liver diseased patients.

3.6 Data collection

3.6.1 Questionnaire interview

A meeting interview was used for filling in a questionnaire which designed for matching the study need for both cases and controls (Annex 1). All interviews were conducted face to face by the researcher himself and the help of the medical

technologists of al Remal clinic. During the survey the interviewers explained any of the questions that were not clear. The questionnaire was based on the questions of previous studies with some modifications **(Jebril, 2012 & Shaat, 2012).** Most questions were the yes/no questions which offer a dichotomous choice **(Backestrom & Hursh-Cesar, 1981)**. The questionnaire includes questions on the personal data (name, age, height, weight, family history of diabetes, smoking and sport) and clinical data including duration of DM and type of drugs (only for patients) and the most important complications of diabetes (retinopathy, numbness in toes, heart diseases, and hypertension).

3.6.2 Body mass index

Body mass index (BMI) was calculated as the ratio of body weight in Kg/height in Meter square. The subjects were asked to remove shoes and heavy clothes before measurement of weight and height. Participant with BMI=18.5–24.9 kg/m^2 was Considered to have normal weight, Participants with BMI=25.0–29.9 kg/m^2 was Classified overweight. Participants with BMI≥30.0 kg/m^2 was considered obese **(WHO, 2000).**

3.6.3 Specimen Collection

Twelve hours fasting overnight venous blood samples were collected from the patients and non diabetic controls. Blood samples (5 ml each) were drawn by the researcher himself and the help of the medical technologists of al Remal clinic on Palestinian medical relief chronic disease clinic and by the team of al Remal clinic's medical technologists in vacationer and plastic tubes from the control and the diabetic patients. About 2 ml blood was placed into EDTA vacationer tube and the remainder quantity of blood (3 ml) was placed in plastic tube and left for a while without anticoagulant to allow blood to clot. Serum samples were obtained by centrifugation at 3000 rpm for 10 minutes for the testing of : glucose, cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), insulin and resistin hormone analysis.

3.7 Biochemical analysis

3.7.1 Determination of serum resistin

Serum resistin was determined by quantitative sandwich enzyme immunoassay technique by using R&D kit, USA.

3.7.1.1 Principle of the assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Resistin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Resistin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Resistin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Resistin bound in the initial step. The color development is stopped and the intensity of the color is measured.

3.7.1.2 Reagents

Description	**Cat. # DRSN00**
Resistin Microplate - 96 Well Polystyrene Microplate (12 Strips Of 8 Wells) Coated With A Mouse Monoclonal Antibody Against Resistin	1 plate
Resistin Conjugate - 21 mL/vial of monoclonal antibody against Resistin conjugated to horseradish peroxidase with preservatives.	1 vial
Resistin Standard - 100 ng/vial of recombinant human Resistin in a buffered protein base with preservatives; lyophilized.	3 vials
Assay Diluent RD1-19 - 11 mL/vial of a buffered protein base with preservatives.	1 vial

Diluents RD5K - 21 mL/vial of a buffered protein base with preservatives.	1 vial
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	1 vial
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	1 vial
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	1 vial
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	1 vial
Plate Covers - Adhesive strips.	4 strips

3.7.1.3 Sample preparation

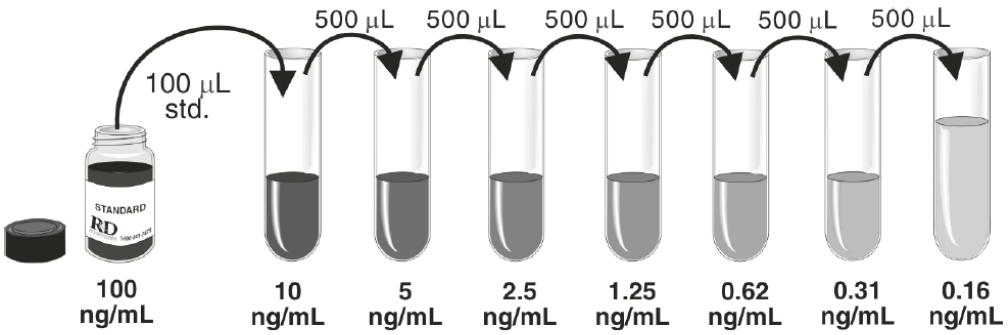
Serum and plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 60 µL sample + 240 µL Calibrator Diluent RD5K.

3.7.1.4 Reagents preparation

Bring all reagents to room temperature before use.

- **Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.
- **Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.
- **Resistin Standard** - Reconstitute the Resistin Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum

of 15 minutes with gentle agitation prior to making dilutions. Pipette 900 µL of Calibrator Diluent RD5K into the 10 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 ng/mL). Prepare fresh for each assay. Use within 4 hours and discard after use.



Serial dilution

3.7.1.5 Assay procedure

Bring all reagents and samples to room temperature before use. Samples, controls and standards were assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previoussections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-19 to each well.
4. Add 100 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 200 µL of Resistin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light.
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a micro plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

3.7.1.6 Quality control of resistin hormone kit

Statistically significant number of controls was assayed to establish the mean values and acceptable range to assure proper performance and the validity of samples results according to control range.

3.7.1.7 Calculation of the results

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Plot the optical density for the standards versus the concentration of the standards and draw the best curve.
3. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.
4. To determine the Resistin concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding Resistin concentration.
5. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

3.7.2 Determination of serum insulin

insulin Serum was determined by quantitative sandwich enzyme immunoassay technique by using DRG Kit ,Germany.

3.7.2.1 Principle of the test

The DRG Insulin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The micro titer wells are coated with a monoclonal antibody directed towards a unique antigenic site on the Insulin molecule.

An aliquot of patient sample containing endogenous Insulin is incubated in the coated well with enzyme conjugate, which is an anti-Insulin antibody conjugated with Biotin. After incubation the unbound conjugate is washed off. During the second incubation step Streptavidin Peroxidase Enzyme Complex binds to the biotin-anti-Insulin antibody. The amount of bound HRP complex is proportional to the concentration of Insulin in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of Insulin in the patient sample.

3.7.2.2 Reagents

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-Insulin antibody (monoclonal).
2. Zero Standard, 1 vial, 3 mL, ready to use 0 µIU/mL Contains non-mercury preservative.
3. Standard (Standard 1-5), 5 vials, 1 mL, ready to use; Concentrations: 6.25 - 12.5 – 25 - 50 and 100 µIU/mL The standards are calibrated against international WHO approved Reference material NIBSC 66/304.; Contain non-mercury preservative.
4. Enzyme Conjugate, 1 vial, 5 mL, ready to use, mouse monoclonal anti-Insulin conjugated to biotin; Contains non-mercury preservative.
5. Enzyme Complex, 1 vial, 7 mL, ready to use, Streptavidin-HRP ComplexContains non-mercury preservative.
6. Substrate Solution, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
7. Stop Solution, 1 vial, 14 mL, ready to use, contains 0.5 M H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.
8. Wash Solution, 1 vial, 30 mL (40X concentrated), see "Preparation of Reagents".

3.7.2.3 Reagent Preparation

- Bring all reagents and required number of strips to room temperature prior to use.
- Wash Solution
- Add deionized water to the 40X concentrated Wash Solution.
- Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
- The diluted Wash Solution is stable for 2 weeks at room temperature.

3.7.2.4 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 25 µL of each Standard, control and samples with new disposable tips into appropriate wells.
3. Dispense 25 µL Enzyme Conjugate into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for 30 minutes at room temperature.
5. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing Procedure.
6. Add 50 µL of Enzyme Complex to each well.
7. Incubate for 30 minutes at room temperature.
8. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add 50 µL of Substrate Solution to each well.
10. Incubate for 15 minutes at room temperature.
11. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.
12. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microliter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

3.7.2.5 Calculation of the Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 µIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

3.7.3 Determination of serum glucose

Serum glucose was determined by glucose oxidase (GOD)/glucose peroxidase (POD) method **(Trinder,1969)** using Daisys Kit, Germany

3.7.3.1 Principle

Determination of glucose after enzymatic oxidation by glucose oxidase. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase.

$$\text{Glucose} + O_2 \longrightarrow \text{Gluconic acid} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \longrightarrow \text{Quinoneimine} + 4\ H_2O$$

3.7.3.2 Reagents

Phosphate buffer (pH 7.5)	25 mmol/l
Phenol	0.5 mmol/l
4-Aminoantipyrine	5 mmol/l
Glucose oxidase (GOD)	≥ 15 ku/l
Peroxidase (POD)	≥ 1 ku/l
Standard	100 mg/dl

3.7.3.3 Assay procedure

About 0.25 ml of serum was transferred to the Mindary Bs-200 Chemistry Auto analyzer, to perform the test according to these parameters:

Reagent volume (µl)	250
Serum volume (µl)	25
Calibrator 1 (mg/dl)	2.7
Incubation time (s)	60
Wavelength (nm)	510
Calibrator type	Multi-points
Measurement Type	End point

3.7.3.4 Calculation

$$\text{Glucose [mg / dl]} = \frac{\Delta A \text{ sample} \times \text{concentration of standard}}{\Delta A \text{ standard}}$$

Reference value (fasting glucose) Adult: 75-115 mg/dl

3.7.4 Determination of serum cholesterol

Serum cholesterol was determined by cholesterol oxidase (COD)/POD method **(Meiatlini et al., 1978)** using Globe kit, Italy

3.7.4.1 Method procedure

The measurement is based on the following enzymatic reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{hydroxybenzoate} + \text{4-Amminoantipyrine} \xrightarrow{\text{POD}} \text{Red complex} + 4H_2O$$

The intensity of the red complex is proportional to the total cholesterol present in the sample.

3.7.4.2 Reagents

Good buffer, pH 6.7	50 mmol/l
Cholesterol oxidase (CHOD)	≥ 100 U/l
Cholesterol esterase (CHE)	≥ 300 U/l
Hydroxybenzoic acid	12 mmol/l
4-Amminoantipirine	0.3 mmol/l
Peroxidase (POD)	≥ 500 U/l
Sodio azide	≤ 0.095 g/l
STANDARD 1x5 ml: Cholesterol	 200 mg/dl

3.7.4.3 Assay procedure

About 0.25 ml of serum was transferred to the Mindary Bs-200 Chemistry Auto analyzer, to perform the test according to these parameters:

Reagent volume (µl)	300
Serum volume (µl)	250
Calibrator 1 (mg/dl)	2.5
Incubation time (s)	60
Wavelength (nm)	510
Calibrator type	Multi-points
Measurement Type	End point

3.7.5 Determination of serum triglycerides

Serum triglyceride was determined by Glycerol phosphate oxidase/peroxidase method **(Bucolo and David, 1973)** using BioSystems kit, Spain.

3.7.5.1 Principle

Triglycerides in the sample onginates, by means of the coupled reactions described below colored complex that can be measured photometrically.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyaceton phosphate} + H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine} + \text{4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + \text{HCI} + 4H_2O$$

3.7.5.2 Reagents

Good Buffer pH 7.2	50 mmol/l
ESPT	4 mmol/l
ATP	2 mmol/l

Mg++	2 mmol/l
Lipoproteinlipase (LPL) [3]	1 kU/l
Glycerol kinase (GK) [3]	0.4 kU/l
Glycerolphosphate oxidase (GPO) [3]	1.5 kU/l
4-Amminoantipirine	0.5 mmol/l
Peroxidase (POD)	>1 kU/l
NaN3	< 0.095 g/l

3.7.5.3 Procedure

About 0.25 ml of serum was transferred to the Mindary Bs-200 Chemistry Auto analyzer, to perform the test according to these parameters:

Reagent volume (µl)	300
Serum volume (µl)	250
Calibrator 1 (mg/dl)	2.5
Incubation time (s)	60
Wavelength (nm)	510
Calibrator type	Multi-points
Measurement Type	End point

3.7.5.4 Calculation

$$\text{Triglycerides [mg / dl]} = \frac{\Delta A \text{ sample} \times \text{concentration of standard}}{\Delta A \text{ standard}}$$

Reference value

Adult (desirable) M 40 - 160 mg/dl

3.7.6 Determination of serum High density lipoprotein cholesterol

HDL-C was determined by precipitating method **(Grove, 1979)** using daisys kit, Germany

3.7.6.1 Principle

Chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and

magnesium ions to the sample. Centrifugation leaves only the HDL in the supernatant, their cholesterol content is determined enzymatically using cholesterol reagent.

3.7.6.2 Reagents

Monoreagent contain: Magnesium chloride	1.4 mmol/l
Phosphotungstic acid	8.6 mmol/l
Choesterol standard	200 mg/dl

3.7.6.3 Assay procedure

1. **Precipitation**

- 200 µl of standard (sample or control) was added to 500 µl of the precipitation reagent and mixed well.
- The mixture was allowed to stand for 15 min at room temperature, and then centrifuged for 20 min at 4000 rpm.34

2. **Cholesterol determination**

Wavelength: 500 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against reagent blank.

100 µl of the supernatant of standard (sample or control) was added to 1 ml of the cholesterol reagent and mixed well.

The mixture was incubated for 5 min at 37 ºC.

The absorbance was measured within 45 min.

3.7.6.4Calculation

$$\text{HDL-C [mg/dl]} = \frac{\text{(A) Test x (C) Standard}}{\text{(A) Standard}}$$

Reference value = 35-65 mg/dl (PCLTG, 2005)

3.7.7 Determination of serum low density lipoproteins

LDL-C can be calculated using the empirical relation of **(Friedewald et al., 1972)**.

3.7.7.1 Principle

The ultra-centrifugation measurement of LDL-C is time consuming and expensive and requires special equipment. For this reason, LDL-C is most commonly estimated from quantitative measurements of total and HDL-C and triglycerides (TG) using the empirical relationship of Friedewald as follows:

LDL-C = Total Cholesterol - HDL-C - TG/5.

3.8 Data Analysis

Statistical Package for Social Sciences (SPSS) software version 19 was used in summarizing, tabulation and analyzing the data. Several statistical procedures have been used for that purpose such as: Pearson correlation coefficient to find relationship between quantitative variables, frequency and descriptive analysis, parametric tests like independent samples T-test and analysis of variance. The independent samples T-test was used to examine if there is statistical significant difference between two means among the respondents. The *One- Way Analysis of Variance (ANOVA)* was used to examine if there is statistical significant difference between several means among the respondents. Results were presented in tables and graphs.

Chapter 4

Results

4.1 Characteristics of the study population

The sample size of the study population is presented in table 4.1 was consisted of 90 subjects, 45 were type 2 male diabetic patients and 45 were controls. The controls matched the cases in sex, age and residence.

Table 4.1. Sample size of the study population

	Frequency	Percent
Controls	45	50%
T2DM	45	50%
Total	90	100%

4.1.2 Age of the study population

The age range of the study population was 40 to 60 years as shown in Table (4.2). The mean age for patients was 51.64 ± 5.74 years. The mean age for control group was 50.93 ± 4.76 years. The independent sample t-test showed no significant difference between mean ages of controls and cases (P=0.524).

Table 4.2. Age of the study population

	Controls		T2DM		p- value by T. test
Age years	Mean	Std. Deviation	Mean	Std. Deviation	
	50.93	5.74	51.64	4.76	0.524

P<0.05: significant

4.1.3 Distribution of the study population with respect to DM duration

Classification of cases according to duration of DM is presented in table 4.3. The first category is less than 5 years and it represented 60% of cases and the second category is for 5 years or more and it represented 40%.

Table 4.3. Distribution of the study population with respect to DM duration

T2DM duration	T2DM	
< 5 year	Count	27
	%	60%
≥ 5 year	Count	18
	%	40%
Total	Count	45
	%	100.0%

4.1.4 Distribution of the study population with respect to Family history of T2DM

Regarding to family history Table.4.4 indicates that 24 (53.3%) of controls and 34 of (75.6%) cases had family history of type 2 diabetes where as 21 (46.7%) and 11 (24.4%) had not. The difference between the two groups was significance (P=0.023), indicating that family history is associated with type 2 diabetes.

Table 4.4. Family history of T2DM

Family history of T2MD	Controls		T2DM		p- value by χ^2
	Frequency	Percent %	Frequency	Percent %	
Yes	24	53.3	34	75.6	0.023
No	21	46.7	11	24.4	

P<0.05: significant

4.1.5 Self-reported complications of case group

Figure 4.2 shows the main self-reported complications among diabetic patients.

The percentages of hypertension were 28.88%, numbness in toes were 31.11%, heart diseases were 17.77% and retinopathy in patient were 35.55%.

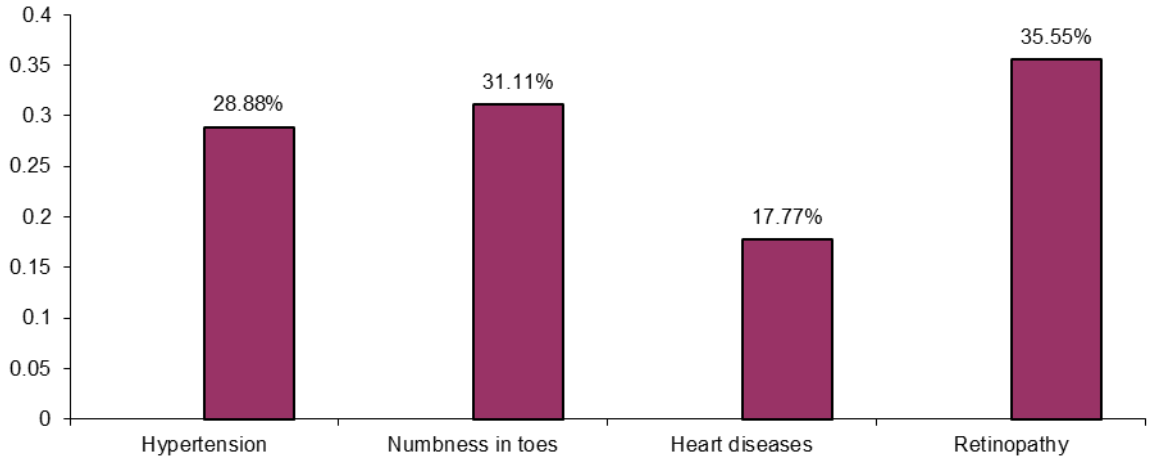


Figure.4.2 Self- reported complications of the case group

4.1.6 Smoking among study population

Table 4.5 shows that there was no statistically significance between controls and T2DM patients in smoking (P=0.50). In controls, 15.6% were smokers while 84.4% were nonsmokers. Among patients 17.8% were smokers and 82.2% were non-smokers.

Table 4.5. Smoking among study population

Smoking	Controls		T2DM		p- value by χ^2
	Frequency	Percent %	Frequency	Percent %	
Yes	7	15.6	8	17.8	0.50
no	38	84.4	37	82.2	

P<0.05: significant

4.1.7 Physical activity of study population

Table 4.6 reveals that There was a statistically significant difference between controls and T2DM patients with respect to physical activity (P=0.023). Among controls 75.6% of answers were yes for the question of practicing sport while 24.4% of answers were no for the same question. For T2DM patients, 53.3% of answers were yes for the same question while 46.7% of answers were no.

Table 4.6. Physical activity among study population

Physical activity	Controls		T2DM		p- value by χ^2
	Frequency	Percent %	Frequency	Percent %	
Yes	34	75.6	24	53.3	0.023
No	11	24.4	21	46.7	

P<0.05: significant

4.1.8 Type of treatment among case group

This figure 4.3 presents the types of treatments used by T2DM patients. The results showed that 2.20% were going on diet, 26.60% were using medical tablets, 11.10% were using both insulin and medical tablets, 37.70% were using tablets and making diet, 6.60% were using insulin and diet, 4.40% were using insulin, diet and medical tablets & 11.10% were without any treatment.

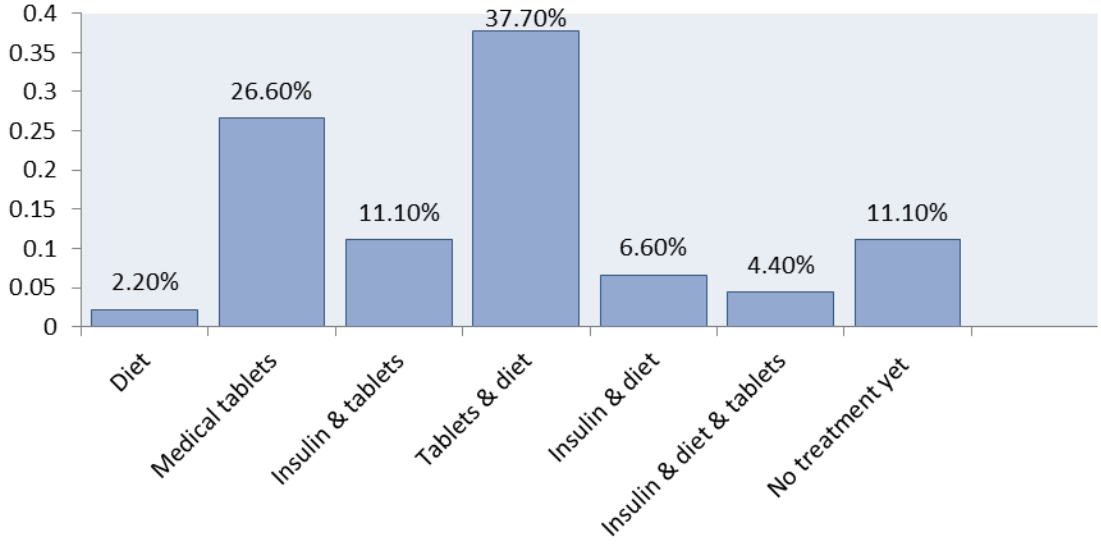


Figure 4.3 Type of treatment among case group

4.1.9 Body mass index (BMI) of the study population

Table 4.7 shows that there was no significant difference in the mean BMI of T2DM patients compared to control (31.81±4.49 vs. 29.75±4.49kg/m^2) (P=0.058).

Table 4.7. Body mass index of the study population

BMI ($Kg/m^{2)}$	Controls		T2DM		p- value by T. test
	Mean	Std. Deviation	Mean	Std. Deviation	
	29.75	4.49	31.81	5.65	0.058

BMI: Body mass index (Kg/m2): People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight, people with BM≥30.0 were considered obese **(WHO, 2000).**
P<0.05: significant

4.2. Biochemical analysis

4.2.1 Serum resistin level among the study population

Table 4.8 shows the mean level of serum resistin among the study population. There was no statistically significant difference in the mean resistin of T2DM compared to controls (11.18±4.59 Vs 10.53±4.59 ng/mL) (P=0.506).

Table 4.8. Serum Serum resistin level among the study population

Resistin ng/mL	Controls		T2DM		p- value by T. test
	Mean	Std. Deviation	Mean	Std. Deviation	
	10.53	4.59	11.18	4.59	0.506

P<0.05: significant

4.2.2 Serum glucose level among the study population

As indicated in Table 4.9 the mean serum glucose levels in T2DM patients were significantly higher than that in controls (180.78±76.3 vs. 90.89±12.88 mg/dl) (P=0.000).

Table 4.9. Serum glucose level among study population

Glucose mg/dl	Control		T2DM		p- value by T. test
	Mean	Std. Deviation	Mean	Std. Deviation	
	90.89	12.88	180.78	76.03	0.000

P<0.05: significant

4.2.3 Serum insulin & HOMA-IR level among study population

Serum insulin & insulin resistant (IR) level of the study population are presented in Table 4.10. There were significant increasing in serum insulin concentration and insulin resistant in cases (15.26±12.87 mg/dl and 6.34±5.75) compared to controls (9.30±5.02 and 1.91 mg/dl ±.076). With (p=0.005 and p=0.000).

Table 4.10. Serum insulin & HOMA-IR level among study population

Parameters	Controls		T2DM		p- value by T. test
	Mean	Std. Deviation	Mean	Std. Deviation	
Insulin Mg/dl	9.30	5.02	15.26	12.87	0.005*
HOMA-IR**	1.91	0.76	6.34	5.75	0.000*

*P value of t-test: P<0.05: is significant

**HOMA-IR: Homeostatasis Model Assessment for insulin resistance insulin resistant

4.2.4 Lipid profile of the study population

Table 4.11 presents serum lipid profile including cholesterol, triglycerides, HDL-C and LDL-C of the study population. There were no different significance in means of cholesterol, triglycerides, HDL-C and LDL-C in controls (204.71±39.47, 170.44±77.24, 50.11±5.14 and 122.33±38.15 mg/dl, respectively) and in cases (203.18±52.82, 180.82±85.28, 48.64±6.18 and 118.67±52.40 mg/dl, respectively) (P=0.876, P=0.547, P=0.224 and P=0.705 mg/dl, respectively).

Table 4.11. Lipid profile of the Study population

Parameters	Control		T2DM		p- value by T-test
	Mean	Std. Deviation	Mean	Std. Deviation	
Total cholesterol mg/dl	204.71	39.47	203.18	52.82	0.876
Triglycerides mg/dl	170.44	77.24	180.82	85.28	0.547
HDL-C* Mg/dl	50.11	5.14	48.64	6.18	0.224
LDL-C mg/dl**	122.33	38.15	118.67	52.40	0.705

*HDL—C : Height density lipoprotein cholesterol
**LDL-C: low density lipoprotein cholesterol
P value of t-test: P<0.05:is significant

4.2.5 Serum resistin compared with family history of the study population

Table 4.12 shows that there was no association between serum resistin level and family history of T2DM in controls and in cases (P=0.641,P=0.191).

Table 4.12. Serum resistin compared with family history of the study population

Family history	**Controls**						
		Group Statistics			**Independent Samples Test**		
		N	Mean	Std. Deviation	t	df	p- value
	yes	24	10.8333	5.55500	0.470	43	0.641
	no	21	10.1833	3.24586			
	T2DM						
		Group Statistics			**Independent Samples Test**		
		N	Mean	Std. Deviation	t	df	p- value
	yes	34	11.6882	4.58610	1.329	43	0.191
	no	11	9.5918	4.41484			

4.2.6 Serum resistin compared with physical activity & smoking among study population

As shown in table 4.13 physical activity or smoking had no significant association with the level of serum resistin among controls or among cases (P>0.05).

Table 4.13. Association of serum resistin with physical activity & smoking among study population

Controls							
Resistin	**Group Statistics**				**Independent Samples Test**		
		N	Mean	Std. Deviation	t	df	p- value
Physical activity	yes	34	9.8074	3.34164	-1.914-	43	0.062
	no	11	12.7636	6.95734			
Smoking	yes	7	10.7571	5.61140			
	no	38	10.4882	4.45949			

T2DM							
Resistin	**Group Statistics**				**Independent Samples Test**		
		N	Mean	Std. Deviation	t	df	p- value
Physical activity	yes	24	11.1983	4.26395	0.035	43	0.972
	no	21	11.1500	5.03639			
Smoking	yes	8	10.6875	5.81278	0.329	43	0.744
	no	37	11.2814	4.36737			

4.2.7 Serum resistin correlated with age among study population

Table 4.14 shows that by using Pearson correlation test there was no correlation between serum resistin and age in controls or in cases (P>0.05)

Table 4.14. Serum resistin correlated with age among study population

Resistin level		Controls	T2DM
Age	Pearson Correlation	0.182	0.101
	P-value	0.231	0.511

: P<0.05: is significant

4.2.8 Correlation of Serum resistin with BMI among study population

Table4.15 shows that there was no correlation between serum resistin level and BMI in controls or in cases (P>0.05).

Table 4.15. Correlation of Serum resistin with BMI among study population

Resestin level		Controls	T2DM
BMI kg/m2*	Pearson Correlation	-.197-	.012
	P-value	.195	.940

BMI: Body mass index

P<0.05: is significant

4.2.9 Correlation of resistin level with serum glucose level

Table 4.16 shows that there was no correlation between serum resstin level and serum glucose in controls or in cases (P=0.590, P=0.750).

Table 4.16. Correlation of resistin level with serum glucose level

Resistin level		Controls	T2DM
Glucose	Pearson Correlation	.084	.049
	P-value	.590	.750

P<0.05:is significant

4.2.10 Correlation of serum resistin with serum insulin & insulin resistance among the study population

Table 4.17 shows that there was no correlation between serum resstin level and serum insulin or insulin resistant in controls or in cases (P>0.05).

Table 4.17. Correlation of serum resistin with serum insulin and insulin

Resistin level		Controls	T2DM
Insulin	Pearson Correlation	.018	0.134
	P-value	0.908	0.378
IR*	Pearson Correlation	0.156	0.098
	P-value	0.307	0.523

*IR:insulin resistant

4.2.11 Correlation of serum resistin with lipid profile among the study population

Table 4.18 shows that there was a negative significant correlation between serum resistin level and total cholesterol and triglyceride in controls. In the other hand there was no correlation between serum resistin level and HDL-C or LDL-C in controls. In the case of T2DM patients serum resistin level has no correlation with cholesterol or triglyceride or HDL-C or LDL-C.

Table 4.18. Correlation of serum resistin with lipid profile among the study population

Parameters		Controls	T2DM
Total cholesterol	Pearson Correlation	-0.328	0.267-
	P-value	0.028	.076
Triglycerides	Pearson Correlation	-0.356	0.035
	P-value	0.016	0.822
HDL-CL*	Pearson Correlation	0.228	0.194
	P-value	0.132	0.202
LDL-CL**	Pearson Correlation	0.133	0.293
	P-value	0.383	.051

*HDL—Cl : Height density lipoprotein cholesterol
**LDL-Cl: low density lipoprotein cholesterol
P value of t-test: P<0.05:is significant

Chapter5

Disscution

5.1 Overview

Diabetes mellitus is a common, serious metabolic disorder with diverse causes and multiple complications **(McCowen & Smith., 2013).** Diabetes affects more than 177 million people worldwide, and its prevalence continues to grow. It is estimated by the World Health Organization that over 300 million people will be affected by diabetes by 2025, of which approximately 90% will be T2DM. As a continuous progressive metabolic disease, type 2 diabetes often begins with years of asymptomatic insulin resistance, which results in hyperglycemia and compensatory hyperinsulinemia **(Chao & Liu., 2008).**

Adipose tissue is known to produce a vast array of adipocyte-derived factors, known as adipocytokines. Under 'normal' physiological conditions adipocytokines may play an influential role in energy homoeostasis, triacylglycerol (triglyceride) storage and mobilization of fat, with increased adiposity, specifically central adiposity. These processes can be substantially dysregulated. Furthermore, it seems apparent that the pathogenesis of T2DM (Type II diabetes mellitus) is mediated through the concurrent progression of insulin resistance and subclinical inflammation, although the molecular mechanisms for this are less understood. It is, however, apparent that obesity represents one of the foremost contributory factors leading to diabetes, as such, the expression and functional properties of adipocytokines and their effects on metabolism have been the subject of intense research **(Kusminski et al., 2005)**.

Resistin, an adipocyte secreted hormone, has been suggested to link obesity with type 2 diabetes and insulin resistance in rodent models, but its relevance to human diabetes remains uncertain**(Mohammadzadeh et al., 2008).** This study was carried out to assess resistin hormone level among T2DM patients and its relation and association with other chemical and anthropometric parameter.

5.2 Characteristics of the study population

This is a case control study included 90 males subjects, 45 of them were type 2 diabetic patients and the rest of them were 45 healthy non diabetic controls.

5.2.1 Age and family history of the study population

The mean age of cases were 51.64±4.76 years and for controls were 50.93±5.74 years .It was reported that type 2 diabetes mellitus usually develops after the age 40 years **(Rodger, 1991 and Umpierrez et al., 2006).** The prevalence of diabetes was higher among individuals with family history of diabetes. These findings indicate that family history is associated with T2DM. This association not only highlights the importance of shared genes and environment in diabetes but also opens the possibility of formally adding family history to public health strategies aimed at detecting and preventing the disease**.** The presence of a family history of diabetes resulted to an early onset of the disease to the offspring **(Shaath., 2012).** In the present study, in accordance with some previous ones **(Vilarrasa et al., 2005)** it did not reveal any changes in blood resistin concentrations with aging. This contradiction requires farther investigation with large sample size.

5.2.2 Diabetes duration and Self-reported complications

The present study data illustrated that 60% of patients had diabetics since less than 5 years. This finding confirms the idea that type 2 diabetes has long asymptomatic pre-clinical phase which frequently goes undetected. During this period of undiagnosed disease, risk factors for diabetic complications are developing **(Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2003).** The most self reported symptoms among diabetic patients were hypertension, numbness in toes, heart diseases and retinopathy.

5.2.3 Smoking among study population

This study indicated that there was no statically difference between cases and control in smoking. However several researchers suggested a significant association between smoking and diabetes, since smoking is considered as an enhancer for oxidative stress and a leading reason for hypertension. There is also substantial evidence that endothelium- dependent vasodilatation is impaired in smokers, type II diabetics, and in subjects with essential hypertension **(Toledo et al., 2004).** In contrast, the present study revealed that there was no association between resistin level and smoking. That required further investigation with larger sample size.

5.2.4 Physical activity among study population

In this study there was a statically significant difference between controls and cases related to physical activity (P=0.023).This insures the role of physical activity in the prevention of diseases like T2DM. Although physical activity (PA) is a key element in preventing and management of T2DM, many with this chronic disease do not become or remain regularly active **(American College of Sports Medicine and the American Diabetes Association, 2010).**

Physical activity can influence the role of insulin in glucose entry into skeletal muscle by causing an insulin independent increase in the number of GLUT-4 transporters in muscle cell membrane. An increase in glucose entry persists for several hours after exercise and regular exercise training can produce prolonged increase in insulin sensitivity **(Ganong 2005).** This present study also revealed that there was no any association between resistin level and sport.

5.2.5 Type of diabetic treatment among cases group

The present study summarized the types of treatment used by T2DM patients. The most used treatment were tablets and diets together, then medical tablets alone. There were few patients not using these treatments because most of them hadn't discovered the occurrence of T2DM yet.

5.2.6 Body mass index

BMI is a measurement that compares individual's weight for height (kg/m^2), and is usually used to assess obesity. Therefore, obesity is commonly defined as a BMI of 30 Kg/m2 or higher. This definition distinguishes obesity from being pre-obese or overweight, which is classified as a BMI of 25 kg/m2 but less than 30 Kg/m2 **(WHO, 2000).** In this study there was no significant difference between control and cases in BMI. It is expected; therefore that BMI should correlate with blood glucose levels. This is, however, not always the case. A Scottish study has previously shown no significant correlation between random blood sugar levels and BMI **(Janghorbani et al., 1991).** Racial and other biological factors may be responsible for this difference as was suggested in a study involving Caucasian and African-American women **(Dowling & Pi-Sunyer., 1993).** As an indicator of adipocity BMI was tested for its correlation with resistin hormone level and because resistin is an adipocyte-secreted hormone, it appears reasonable to expect that it should be related to whole body or regional body adiposity. However, many human studies (**Savage et al., 2001, lee et al., 2003, McTernan et al., 2003, Yang et al., 2003)** and this one have not found any evidence of this relationship. In contrast Some studies **(Azuma et al., 2003, Degawa et al., 2003, Fujinami et al., 2004)** have reported a positive correlation between resistin level and BMI, and one particular group has demonstrated that the resistin level is associated positively with total body fat **(Yannakoulia et al., 2003).**

Previous reports implied that adipocytes are not the only source of resistin hormone and, in fact, may not be the major source of resistin secretion in human beings. Such finding may explain why no relationship between resistin level and adiposity has been noted **(Ching-Chu et al., 2005).**

5.3 Biochemical analysis

5.3.1 Serum resistin level among study population

The present finding shows that there was no significant difference in the level of serum resistin between controls and cases. There are also a number of studies indicated that there is no significant difference between controls and T2DM patients with respect to resistin level **(Lee et al. 2003, Stejskal et al. 2003, Yaturu et al. 2006 & Norata et al. 2007)**. However, there are an almost equal number of studies in which statically difference among the study population has been observed **(McTernan et al. 2003, Zhang et al. 2003, Youn et al. 2004, Takata et al. 2008).** A result that it seems depends on the size of sample used in each study. The study of Youn and colleagues was used more than 100 individuals in both groups **(Youn et al. 2004). Schäffler et al., (2004**) has reported that the plasma resistin level is lower in the group of type 2 diabetics (n = 555) compared to healthy controls (n = 216)**.**

5.3.2 Serum glucose level among the study population

As indicated in the present finding, the mean glucose levels in cases were significantly higher than that in controls. Similar results were obtained by **Qi et al., (2007) and Yassin et al. (2011)** who found that T2DP had higher fasting glucose levels than non-diabetics. In diabetes, prolonged hyperglycemia super drives nonenzymatic protein glycation, which forms reversible Schiff bases and Amadori compounds. A series of further complex molecular rearrangements then yield irreversible advanced glycosylated end-products (AGEs). AGEs accumulate in the circulating blood and in various tissues **(Furth, 1997)**.

In this study no significant correlation between serum glucose and serum resistin was observed**.** Similarly studies in Pima Indians have reported serum resistin levels were not correlated with fasting glucose and insulin levels **(Vozarova et al., 2004).** Other studies indicated that resistin expression is reduced by fasting and hence low insulin level and increases rapidly on re-feeding and hence high insulin level **(Steppan et al., 2001).**

5.3.3 Serum insulin and insulin resistant level among study population

The findings of this study revealed that serum insulin and IR mean level in T2DM was higher than those in controls. These results confirm the role of insulin hormone and IR in T2DM. Many people with IR have high levels of both glucose and insulin circulating in their blood at the same time. However, insulin resistance increases the chance of developing T2DM and heart disease **(National Diabetes Information Clearinghouse. 2008).**

In its correlation with resistin hormone this study didn't find any possible correlation between insulin hormone or IR. similarly other studies have reported no associations between serum resistin levels and markers of insulin resistance among T2DM patients **(Lee et al., 2003 and Stejskal et al., 2003)** or insulin-resistant patients **(Hegele et al., 2003).** Moreover, serum and plasma resistin levels were either reduced or increased in T2DM patients with no significant correlation with HOMA-IR**.** In contrast, Silha et al., (2003) a reported a significant correlation between resistin and HOMA-IR. With regard to diabetes, a number of studies have described higher circulating resistin levels in diabetic as compared with non-diabetic subjects. This increase was not associated with markers of IR or adiposity**(Zhang et al., 2003 and McTernan et al., 2003)** other studies in contrast reported that serum resistin concentrations were not significant different between diabetic and non-diabetic obese subjects. This contraelection in the finding about correlation of resistin with insulin or IR required further investigation

5.3.4 Lipid profile of the study population

Data presented in this study revealed no significant difference in the mean levels of cholesterol, triglycerides, HDL-C and LDL-C in diabetic patients compared to controls. This study demonstrated that total cholesterol and triglycerides is negatively colerrated with resistin hormone in control group only. Another study has revealed that the serum resistin level is negatively associated with HDL-C level for both sexes. However, this relationship exists only when the data are analyzed by multiple linear regression and log resistin in female subjects. The biologic and physiological significance of this finding therefore needs to be proven. Jove et al (2003) reported the existence of a negative correlation between resistin mRNA level from white adipose tissue, total cholesterol, and LDL-C levels in 8 subjects subsequent to fenofibrate treatment. Those authors concluded that cholesterol regulated resistin expression within human white adipose

tissue. Other studies **(Lee et al., 2003, Pfutzner et al., 2003 and McTernan et al., 2003)** however, similar to this finding current study, has not observed any relationship between resistin level and total cholesterol and LDL-C levels **(Ching et al., 2005).** In other studies, it has been demonstrated that resistin is associated with low HDL in healthy and T2DM (diabetes mellitus type 2) subjects **(Eckel et al., 2005 and Ching et al., 2005)**. Mice, of which resistin is over-expressed in the liver by adenovirus, have the characteristics of Metabolic Syndrome such as increased insulin resistance, low serum HDL and high triglyceride **(Sato et al., 2005).** Insulin is known to up-regulate lipoprotein lipase, a critical factor for the production of HDL, and the lipolysis of triglyceride, and this relation could be the explanation of this association **(Luis et al., 2011).**

Chapter 6
Conclusions and Recommendations

6.1 Conclusions

- The mean ages of controls and cases were 50.93±5.74 and 51.64±4.76 years, respectively.
- Diabetes mellitus is more prevalent among individuals with family history of diabetes.
- Most of studied patients had diabetes since less than 5 years.
- The self-reported complications among diabetic patients were hypertension, numbness in toes, heart diseases and retinopathy.
- Smoking had no association with serum resistin level.
- Physical activity had no association with serum resistin level.
- The BMI hadn't any possible correlation with serum resistin levels.
- The mean level of serum resistin had not any significant difference between diabetic patients and controls.
- The mean serum glucose levels in cases were significantly higher than that in controls, and there was no any significant correlation with resistin.
- The mean serum insulin an insulin resistant levels in cases were significantly higher than that in controls, with no any significant correlations with resistin.
- The average level of cholesterol, triglycerides, HDL-C and LDL-C had not significantly difference in cases compared to controls. The Pearson correlation test revealed negative significant correlation between resistin and triglyceride or cholesterol.

6.2 Recommendation

- Further studies are recommended with large sample size to investigate the relation of resistin with IR and insulin level.
- Researches on resistin hormone in a type of cell culture research are recommended.
- Investigation of the association of hormone resistin with other diseases is also recommended.

Chapter 7
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Annex 1

Questionnaire

السادة الكرام

السلام عليكم ورحمة الله ،،،،

أنا الباحث مؤنس مروان الهندي (طالب ماجستير تحاليل طبية بالجامعة الإسلامية) أرجو المساعدة في إتمام هذه الدراسة وذلك من خلال تعبئة هذا الاستبيان وتبرعك لنا بعينة دم لإجراء بعض الفحوصات الطبية.

شاكرين لكم حسن تعاونكم

(الاســم اختياري) : ..... **رقم الجوال** :.....

العمـــر : .....

الطــول: .....سم ، **الـــوزن:** .....كجم

BMI :.....

1. **منذ متى تعاني من مرض السكري؟**

☐ لا أعاني من مرض السكري

☐ أقل من 5 سنوات ؟ حدد عدد السنوات .....

☐ أكثر من 5 سنوات ؟ حدد عدد السنوات.....

2. هل يعاني أحد أفراد عائلتك من مرض السكري من النوع الثاني ؟

نعم ☐ لا ☐

- **إذا كانت الإجابة نعم : ماهي درجة القرابة ؟.....**

3. هل تعاني من مشاكل صحية أو أمراض ؟؟

نعم ☐ لا ☐ إذا كانت الإجابة نعم اذكرها ؟

☐ ارتفاع ضغط الدم ☐ تنميل في أصابع القدم

☐ مشاكل في القلب ☐ مشاكل في العيون

☐ غير ذلك (حدد).....

4. هل تقوم بفحص السكر في الدم لديك بشكل دوري؟

نعم ☐ لا ☐

- **إذا كانت الإجابة نعم:**
- **متى أخر مرة قمت بفحص السكر؟.....**
- **ما هو معدل السكر في أخر فحص قمت به ؟.....**

5. ما هو نوع العلاج الذي تستخدمه في الوقت الحالي ؟

- **حمية غذائية**
- **أقراص دواء عن طريق الفم .**
- **أنسولين**
- **جميعهم**

6. إذا كنت تتبع الحمية الغذائية فهل أنت ملتزم بها تماما ؟

نعم ☐ لا ☐

7. إذا كنت تستخدم أقراص دواء عن طريق الفم:
فهل تتناول هذه الأقراص بانتظام ؟

نعم ☐ لا ☐

- **ما هو اسم الأقراص التي تستخدمها ؟ .....**

8. إذا كنت تستخدم الأنسولين كعلاج فهل أنت مواظب على أخذ العلاج بالجرعة المحددة التي وصفها لك الطبيب وفي الوقت المحدد لها ؟

نعم ☐ لا ☐

9. هل حدث تغيير في كمية الأنسولين المستخدم خلال الخمس سنوات الأخيرة ؟

نعم ☐ لا ☐

- **ما هو حجم التغيير؟.....**

10. هل حدث تغيير في نوع العلاج المستخدم خلال الخمس سنوات الأخيرة ؟

نعم ☐ لا ☐

- **إذا كانت الإجابة نعم : ما هو التغيير الذي حدث ؟.....**

11. هل تمارس أي نوع من أنواع الرياضة ؟

نعم ☐ لا ☐

- **إذا كانت الإجابة نعم : ما هو نوع الرياضة التي تمارسها و كم مدتها؟.....**

12. هل تمارس عادة التدخين ؟

نعم ☐ لا ☐

- **إذا كانت الإجابة نعم : منذ متى وأنت تدخن ؟ .....ز**

13. هل تعاني من ارتفاع ضغط الدم ؟

نعم ☐ لا☐

- **إذا كانت الإجابة نعم:**
- **منذ متى وأنت تعاني من ارتفاع ضغط الدم ؟ .....**
- **ما هو اسم العلاج الذي تستخدمه لعلاج ارتفاع ضغط الدم؟ .....**

14. **هل تظن أنك تعرف معلومات كافية عن حالتك الصحية و الأدوية التي تأخذها؟**

نعم ☐ لا ☐

15. **هل تستشير أكثر من طبيب لمعالجة السكري؟**

نعم ☐ لا ☐

16. كم تملك من الثقة في السيطرة على مرض السكري؟

□ واثق جدا

□ واثق قليلا

□ ليس لدي أي ثقة

17. هل تملك جهاز قياس نسبة السكر في الدم؟

نعم ☐ لا☐

18. كم مرة تقيس نسبة السكر في دمك باستعمال هذا الجهاز ؟

☐عدة مرات في اليوم ☐عدة مرات في الأسبوع ☐ أبداً

- انا موافق على تعبئة هذا الاستبيان الذي يتعلق بصحتي.

التوقيع: .....

التاريخ:.....

شكرا لكم على حسن تعاونكم

الباحث / مؤنس الهندي

Annex 2

The Palestinian National Authority
Ministry of Health
Directorate General of Human Resources Development

السلطة الوطنية الفلسطينية
وزارة الصحة
الإدارة العامة لتنمية القوى البشرية

الرقم :.....

التاريخ: 2013/04/23م

المحترم،،،

الأخ / د. فؤاد العيسوي
مدير عام الرعاية الأولية

وزارة الصحة
الإدارة العامة للرعاية الأولية
2852
2013/4/25

السلام عليكم ورحمة الله وبركاته،،،

الموضوع/ تسهيل مهمة باحث

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحث / مؤنس مروان الهندي الملتحق ببرنامج ماجستير العلوم الحياتية قسم التحاليل الطبية- كلية العلوم - الجامعة الإسلامية غزة في إجراء بحث بعنوان :-

" Resistin Hormone Level among Type 2 Diabetes Mellitus Patient in Gaza Strip "

حيث الباحث بحاجة لتعبئة استبانه وجزء من عينة دم سحبت لإغراض تشخيصية من عدد من مرضى السكر النوع الثاني وأخرى ممن لا يعانون من هذا المرض من المراجعين لعيادات الغدد الصماء في عيادة شهداء الرمال.

كما نأمل توجيهاتكم لذوي الاختصاص بعدم السماح للباحث بالتطبيق إلا بعد الحصول على الموافقة المستبصرة من المشاركين في البحث وبإشراف العاملين في أقسام المختبرات ووفق الأسس التي يتم بها التعامل مع هذا النوع من العينات في الوزارة وعلى مسئولية الباحث، و بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، و دون تحمل الوزارة أي أعباء أو مسئولية.

وتفضلوا بقبول التحية والتقدير،،،

د. ناصر رأفت أبو شعبان
مدير عام تنمية القوى البشرية

وزارة الصحة
تنمية القوى البشرية
صادر
التاريخ

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- صاحب/ـة العلاقة

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